AD-A216 175

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	24 Sep 84	Final (1 Mar 84-28 Feb 85

4. TITLE AND SUBTITLE

RESEARCH PROGRESS AND FORECAST REPORT

5. FUNDING NUMBERS

61102F
2312/A1

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8. PERFORMING ORGANIZATION REPORT NUMBER

AFOSR-TR- 89-1232

SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR
BLDG 410
BAFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

AFOSR-84-0086

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

14. SUBJECT TERMS

15. NUMBER OF PAGES
1 thru 2

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified		

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September 24, 1984

Ms. Louise Harrison
Chief, Support Services Division
Directorate of Contracts
Building 410
Bolling Air Force Base
Washington, D. C. 20332

AFOSR-TR- 89-1532

Re: Grant AFOSR-84-0086

Dear Ms. Harrison:

Enclosed please find a Research Progress and Forecast Report for the abovementioned grant. Dr. William Wallace has left the University and has been replaced by Dr. Ken Mackie. Dr. Robert Lewis has received a postdoctoral fellowship and Dr. Selma Kanazir has taken his place on this project. No major or special equipment has been acquired. Except for the change in commitments of personnel associated with the research effort, there are no anticipated departures or deviations from the planned research approach considered necessary to achieve the stated research objectives.

Please feel free to contact me at 212-570-8780 if you have any questions or require any additional information.

Sincerely yours,

Paul Greengard

Paul Greengard, Ph.D.
Professor and Head
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and Cellular Neuroscience

PG:jh

cc: Leslie McKenzie

DEVELOPMENTAL BIOLOGY OF THE SYNTHESIS OF NEURONAL PHOSPHOPROTEINS

Brains were dissected from rats of various ages from birth to adult and immediately frozen in liquid nitrogen. Cytosolic polysomes from rat brain exclusive of cerebellum were isolated by homogenization and centrifugation through a sucrose shelf. The isolated polysomes were used to direct protein synthesis in a rabbit reticulocyte in vitro translation system in the presence of ^{35}S-methionine. Serum antibodies specific for protein III were added to the translation mixture and precipitated out of solution by the addition of Staphylococcus aureus cells. The protein products were separated by SDS-polyacrylamide gel electrophoresis and subjected to autoradiofluorography. The bands corresponding to protein III were cut from the gel and the incorporated ^{35}S was measured by a scintillation counter. The degree of protein III synthesis correlated well with the amount of synaptogenesis occuring during each developmental stage.

Cytosolic polysomes from the cerebella of rats were isolated and used to direct in vitro translation as described above. Serum antibodies specific for G-substrate were added to the translation mixture and precipitated out of solution by the addition of Staphylococcus aureus cells. The protein products were separated by SDS-polyacrylamide gel electrophoresis and subjected to autoradiofluorography. The band corresponding to G-substrate was scanned with a densitometer and the values for the G-substrate peaks were normalized against the value for postnatal day 20. Synthesis of G-substrate could not be detected before postnatal day 6. The level of synthesis increased slowly from postnatal day 6 to 12, increased rapidly from postnatal day 12 to 24, then decreased to the adult level. There was no correlation between the relative level of G-substrate synthesis and the relative total protein-synthesizing activity of the cerebellar polysomes during development. The period of G-substrate synthesis is concurrent with the period of formation of synapses between Purkinje cells and the other neuronal cells of the cerebellum. The observation that the syntnesis of G-substrate occurs late in the development of Purkinje cells supports the interpretation that this phosphoprotein is involved in a function characteristic of these specialized cells.

MOLECULAR CLONING OF cDNA FOR NEURONAL PHOSPHOPROTEINS

Total poly A+ RNA was isolated from total rat brain by homogenization in guanidine thiocyanate, centrifugation through CsCl and chromatography on oligo dT cellulose. cDNA was synthesized by reverse transcription and ligated into the expression vector pUR250 with EcoRI and BamHI linkers by standard procedures. The colonies were screened for inserts coding for Synapsin I with rabbit serum antibodies specific for Synapsin I and horseradish peroxidase-conjugated goat anti-rabbit IgG serum. Of the 6000 colonies screened, four colonies were positive for Synapsin I synthesis in both the primary and secondary screens. A total protein lysate from each of the four positive colonies was prepared, separated on SDS-polyacrylamide gels and blotted on nitrocellulose paper. The blots were incubated separately with three preparations of rabbit serum antibodies and five preparations of monoclonal antibodies specific for Synapsin I. The antibodies bound to different molecular

weight bands for each of the clones (Clone #1: 12,000 Daltons; Clone #2: 30,000 Daltons; Clone #3: 35,000 Daltons; Clone #4: 40,000 Daltons). All preparations of rabbit serum antibodies and monoclonal antibodies specific to Synapsin I bound to Clone #4. No more than three of the four serum antibody preparations bound to the other three clones. These observations are consistent with the interpretation that these four clones contain inserts for Synapsin I cDNA and are expressing the protein. Clone #4 contains an insert coding for a major portion of the Synapsin I molecule (57,000 Daltons). Clones #1,2 and 3 contain smaller inserts which do not contain all of the antigenic sites recognized by our serum antibodies. Total RNA and poly A+ RNA from total rat brain, partially purified Synapsin I mRNA, and total RNA and poly A+ RNA from lung and spleen were separated on denaturing agarose gels and blotted onto nitrocellulose paper. The nitrocellulose paper was incubated with nick-translated DNA labelled with [α^{32}P]-dCTP generated with plasmid DNA from Clone #4. The probe bound to three bands of 2.7, 1.8 and 1.0 kilobases on the rat brain RNA blot. Only one band, the 2.7 kilobase band, was visible on the blot of partially purified Synapsin I mRNA. No bands were visible on the blots of lung and spleen RNA, although these blots did bind the actin control probe. These results are consistent with the interpretation that the mRNA coding for Synapsin I is represented by the 2.7-kilobase band. The 1.8-and 1.0-kilobase bands are of sufficient size to represent the mRNA's coding for, respectively, protein IIIa and protein IIIb. The fact that the Clone #4 probe also binds to these bands is consistent with previous observations that there may be homologies between Synapsin I and protein III. We are currently determining the sequence of Clone #4 by the dideoxy sequencing method. The section of sequence we currently have is:

5' gly ser arg arg arg gly pro pro leu val ala pro arg pro leu
arg ala leu ala ala leu arg pro ser pro lys gln met met asn
ser glu ala arg leu ser asp 3'

We will compare the sequence of Clone #4 with the fragments of sequence for Synapsin I determined by peptide sequencing to confirm that Clone #4 contains an insert coding for Synapsin I.

We are currently generating libraries of bovine caudate cDNA and rabbit cerebellar cDNA in λGT10, M13 and pUR250. These libraries are being screened with synthetic oligonucleotide probes with sequences that match the the potential sequences coding for DARPP-32 and G-substrate. These sequences were derived from the sequences of fragments of DARPP-32 and G-substrate determined by peptide sequencing.